In claim 125, line one, delete "further."

In claim 126, line one, delete "further."

Remarks

Reconsideration of this Application is respectfully requested.

I. Status of the Claims

Claims 91, 97-103 and 122-126 have been amended. Claims 89-126 are active in this application.

II. Support for the Amendment

In accordance with MPEP § 608.01(v), the Specification has been amended such that the all of the letters in each of the trademarks "ALBUMAX® I and "ALBUMAX II®" are capitalized, and generic terminology for these trademarks has been provided. Support for the generic terminology is found in the Specification at page 15, lines 19-21. Further support for the generic terminology is found at pages 6-25 (for ALBUMAX® I) and 6-26 (for ALBUMAX II®) of the GIBCOBRL Product and Reference Guide 1995-1996 (Life Technologies, Gaithersburg, MD). A copy of each of pages 6-25 and 6-26 is appended hereto, collectively, as Attachment 1.

Support for the word "murine" in claim 97 is found in the specification at page 18, lines 23-30. Mouse cells are murine cells.

Support for the word "supports" in claims 91, 98, 99-103, 122 and 123 is found in the specification at page 3, line 13; page 16, line 1; in claims 22 and 24 as filed originally; and in the Abstract.

No new matter has been added by this amendment.

III. The Form PTO-1449

On page 16 of the Form PTO-1449 filed December 21, 1999, Applicants listed Rose *et al.*, *Cytokine 6*: 48-45 (1994) ("Rose") as document AT16. The Form PTO-1449 contains a typographical error: the pages in Rose should have been listed as "48-54."

Applicants respectfully request that the Examiner correct page 16 of Form PTO-1449 to indicate the Examiner's consideration of pages 48-54 of Rose, and provide Applicants with a copy of the corrected Form PTO-1449.

IV. The Objection To The Specification Must Be Withdrawn

At page 2 of the Office Action, the Examiner objected to the use of the mark "AlbuMax," and explained that the market should be typed in uppercase. The specification has been amended to recite the mark "ALBUMAX." Applicants respectfully request that this objection be reconsidered and withdrawn.

V. The Objections To Claims 97, 125 and 126 Must Be Withdrawn

At page 2 of the Office Action, the Examiner objected to claims 125 and 126. Claims 125 and 126 have been amended to delete the term "further." As a result of the

amendment of claims 125 and 126, it is believed that the ground for this objection is moot. Applicants respectfully request that this objection be reconsidered and withdrawn.

At page 3 of the Office Action, the Examiner objected to claim 97. Claim 97 has been amended to recite "murine cells." As a result of the amendment of claim 97, it is believed that the ground for this objection is moot. Applicants respectfully request that this objection be reconsidered and withdrawn.

VI. The Rejections Under 35 U.S.C. § 112, First Paragraph, Must Be Withdrawn

At page 3 of the Office Action, the Examiner rejected claims 89-126, under 35 U.S.C. § 112, first paragraph, as allegedly not enabled. Applicants respectfully traverse this rejection. A *prima facie* case of non-enablement has not been established.

At page 7 of the Office Action, the Examiner rejected claims 117-121, as allegedly not enabled. Applicants respectfully traverse this rejection. A *prima facie* case of non-enablement has not been established.

A. The Standard for Enablement

The initial burden of proving that a specification is non-enabling is on the Examiner. Under controlling Federal Circuit precedent, it is axiomatic that a specification is presumed to be enabling unless the Examiner provides acceptable objective evidence or sound scientific reasoning showing that it would have required undue experimentation for one of ordinary skill in the art to make and use the claimed invention. In *In re Cortright*, 165 F.3d 1353, 49 USPQ2d 1464, 1469 (Fed. Cir.

1999), the court stated that the PTO cannot make a 112, first paragraph, rejection for lack of enablement, unless the PTO "has reason to doubt the objective truth of the statements contained in the written description." The *Cortright* panel cited *In re Marzocchi*, 439 F.2d 220, 223, 169 USPQ 367, 369 (CCPA 1971). In *Marzocchi*, the court stated:

> [A] specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented *must* be taken as in compliance with the enabling requirement of the first paragraph of § 112 *unless* there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. 169 USPQ 367, 369 (CCPA 1971).

Under *Cortright* and *Marzocchi*, the claims in an application are presumed to be enabled, unless proven otherwise. Further, it is well-established that some experimentation is permitted, so long as it is not "undue." *See In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) ("Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation.") (citations omitted); *see also Ex parte Forman*, 230 USPQ2d 546, 547 (BPAI 1986) ("The ultimate question . . . is whether or not the specification contains a sufficiently explicit disclosure to enable one having ordinary skill in the relevant field to practice the invention claimed therein without the exercise of undue experimentation.").

Thus, the initial burden is not on Applicants to prove that a claimed invention is enabled. Instead, the initial burden is on the Examiner to establish a *prima facie* case of non-enablement, by providing objective evidence or sound scientific reasons why

the claimed invention is allegedly not enabled. As discussed below, the Examiner has not provided reasons sufficient to create a *prima facie* case of non-enablement.

B. With Respect to the Rejection of Claims 89-126, a Prima Facie Case of Non-Enablement Has Not Been Established

1. The Examiner's Proffered Reasons Are Insufficient To Establish a Prima Facie Case of Non-Enablement

At pages 4-5 of the Office Action, the Examiner stated:

> [W]ith regard to the broadly claimed embryonic stem cells, at the time of filing, the state of the art is such that the generation of ES cells, i.e., cells which retain their totipotential capacity and are able to generate cells of all lineages, including germline, after being introduced into host a [sic] blastocyst, is neither routine nor predictable in species other than mice. Keller (Current Opinion in Cell Biology, 1995) teaches that ES cells are totipotent lines derived from the inner cell mass of developing blastocysts. When maintained on embryonic fibroblasts in culture, ES cells retain their totipotential capacity and are able to generate cells of all lineages, including germline, after being introduced into a host blastocyst (see page 862, left column, first paragraph).
>
> However, at the time of filing, the state of the art is such that generation of ES cells, i.e., cells which retain their totipotential capacity and are able to generate cells of all ages, including germline, after being introduced into host a blastocyst [sic], is neither routine nor predictable in species other than mice. Bradley (Biotechnology, 1992) teaches that while a number of reports have been made claiming isolation of ES cells from farm animals, the description of the cell lines is yet to be supported by the demonstration that they can proliferate and differentiate in an embryo, *in vivo*, contributing to somatic tissues or germ cells (see page 53, right column, last paragraph bridging page 54).[1] Moreover, Seamark (Reproductive Fertility and

[1] The Examiner apparently meant to refer to page 537 of Bradley, last paragraph, bridging to page 538, first paragraph.

> Development, 6:653-657, 1994) discloses that totipotency for ES cell technology in many livestock species has not been demonstrated (see, e.g., Abstract). Similarly, Matsui et al. (Cell, 1992) disclose that while it is well established that pluripotential stem cells, i.e., those originally termed ES cells, can be derived from the epiblast of blastocysts in culture, it is crucial to determine whether blastocyst-derived stem cells differ in their full range of developmental potencies and properties, such as genomic imprinting (see page at 845, right column, 2nd paragraph, under "Reprogramming...", and page 846, left column, second full paragraph). In view of the lack of guidance in the specification with regard to the process of obtaining embryonic stem cells with the requisite "embryonic stem cell properties", the use of cells in the claimed cell culture method and the claimed products of manufacture is not enabled.

At pages 6-7 of the Office Action, the Examiner stated:

> Moreover, the state of the art at the time of filing indicates the difficulties of culturing embryonic stem cells. For example, Baribault *et al.* (Mol. Biol. Med., 1989) disclose that culture conditions and passage number may influence the ability of ES cells to give rise to germ-line chimeras. Different conditions might induce changes in karyotype, or culture of ES cells for many passages may select for the cells with a higher growth rate and teratocarcinoma-like phenotype. Baribault *et al.* also teach that when embryonal carcinoma cells are re-injected into mouse blastocysts, they infrequently produce germ-line chimeras but often induce tumors. A further consideration of culturing ES cells under conditions for maintaining an undifferentiated state is the stability of the stem cell phenotype, which should be monitored using cell surface markers which identify the embryonic phenotype, as well as regular karyotyping (see page 485, left column, first paragraph).
>
> Taken together, the state of the art the time of filing establishes the unpredictability of obtaining embryonic stem cells and maintaining such cells in culture such that the cells retain the requisite "stem cell characteristics", i.e., the ability to proliferate and differentiate in an embryo, *in vivo*, contributing to somatic tissues or germ cells. Inasmuch as the specification only

> discloses one particular medium formulation which supports the growth of specific embryonic stem cell lines, D3 ES cell line, the two mouse strain 129 ES cell lines, E14 and R1, and a non-129 ES cell line, TT2, such that said cells maintain a stable phenotype and retain their totipotential capacity such that they are able to generate cells of all lineages, including germline, after being introduced into a host blastocyst, one of skill in the art would not have a high expectation of making in using the invention of claim without undue experimentation.

As discussed below, the Examiner's proffered reasons are insufficient to establish that the claimed methods, composition, and product of manufacture would not have been enabled using embryonic stem cells other than the specific mouse embryonic stem cells exemplified in the present specification, and using any serum-free medium other than the serum-free medium exemplified in Tables 1-3 (pp. 27-29) of the present specification.

2. Given the Disclosure of the Present Specification, the Claimed Methods Could Have Been Practiced Without Undue Experimentation

a. The Claims Relate to In Vitro Expansion of Embryonic Stem Cells, Not to Germline Transmission of Genetic Information

At page 14 of the present specification, lines 7-10, Applicants define the term "embryonic stem cell" and "pluripotent embryonic stem cell" to "refer to a cell which can give rise to many differentiated cell types in an embryo or an adult, including the germ cells (sperm and eggs)." Nevertheless, the claimed methods, composition and product of manufacture relate to *in vitro* expansion of embryonic stem cells, not to the *in vivo* effect of embryonic stem cells that have been inserted into a host animal blastocyst.

In analyzing whether the claimed methods, composition and product of manufacture are enabled, the Examiner should focus on what is claimed. Here, the Examiner has misinterpreted the claims to include an *in vivo* limitation, such that for a cell to be regarded by those of ordinary skill in the art as an embryonic stem cell, the cell must exhibit transmission of genetic information *in vivo*. However, the claims do not recite such a limitation.

Those of ordinary skill in the art regarded cells from many species as embryonic stem cells, even before genetic transmission experiments were successful. Those of ordinary skill in the art regarded cells as embryonic stem cells by virtue of (1) morphological characteristics, (2) the ability to be maintained in culture in an undifferentiated state, or (3) the ability to control the differentiation of the cells in culture. Once cells were deemed embryonic stem cells, they were tested for their ability to promote germline transmission of genetic information, exhibited by the production of chimeric animals. Even if germline transmission experiments were not successful, the cells were not automatically assumed not to be embryonic stem cells, because technological obstacles to germline transmission may prevent the transmission of genetic information, even from embryonic stem cells.

The Examiner has not provided any evidence or technical reasons why one of ordinary skill in the art would have doubted that embryonic stem cells could have been identified based on (1) morphological characteristics, (2) the ability to be maintained in culture in an undifferentiated state, or (3) the ability to control the differentiation of the cells in culture. It is improper for the Examiner to require that before a cell could have been regarded as an embryonic stem cell, transmission of genetic information *in vivo*

must have been shown. Further, none of the art cited by the Examiner (i.e., Keller, Bradley, Seamark, Matsui or Baribault) cast doubt on the assumption that the claims 89-126 are enabled.

b. Only Routine Experimentation Would Have Been Required to Have Used Embryonic Stem Cells Other Than Those Exemplified in the Present Specification

The Examiner has provided no evidence or sound technical reasons why the methods, composition and product of manufacture, which, *as claimed*, relate to *in vitro* expansion of embryonic stem cells, would allegedly not have been enabled with embryonic stem cells other than the mouse cells exemplified in the present specification.

Indeed, the pre-filing date literature is replete with examples that those of ordinary skill in the art have developed embryonic stem cell lines from multiple strains of mice, and from non-mouse species. Following are such examples from the literature:

Doetschman, T. *et al.*, *Developmental Biology 127*: 224-227 (1988) (Attachment 2) discloses the isolation and culture of hamster embryonic stem cell lines and show that they are highly pluripotent.

Du, F. *et al.*, *J. Reprod. Fertility 104*: 219-223 (1995) (Attachment 3) discloses the isolation and culture of putative rabbit embryonic stem cells, and that nuclear transfer of the cells leads to normal blastocyst development.

Graves, K.H. *et al.*, *Mol. Reprod. Development 36*: 424-433 (1993) (Attachment 4) discloses the isolation and culture of putative embryonic stem cell lines from rabbit.

Iannaccone, P.M. *et al.*, *Developmental Biology 163*: 288-292 (1994) (Attachment 5) discloses the isolation and culture of rat embryonic stem cells, and the production of chimeric rats using rat embryonic stem cells.

Labosky, P.A. *et al.*, *Development 120*: 3197-3204 (1994) (Attachment 6) discloses the culture of mouse embryonic stem cells, and the production of chimeric mice using a mouse embryonic stem cells.

Magin, T.M. *et al.*, *Nucleic Acids Res. 20*: 3795-3796 (1992) (Attachment 7) discloses the isolation and culture of mouse embryonic stem cells, and the production of chimeric mice using mouse embryonic stem cells.

Notarianni, E. *et al.*, *J. Reprod. Fert., Suppl. 41*: 51-56 (1990) (Attachment 8) discloses the isolation, maintenance, and differentiation in culture of pluripotential embryonic cell lines from pig.

Petitte, J. N. *et al.*, U.S. Patent No. 5,340,740, issued August 23, 1994 (Attachment 9) discloses the isolation and culture of chicken embryonic stem cells.

Saito, S. *et al.*, *Roux's Arch. Dev. Biol. 201*: 134-141 (1992) (Attachment 10) discloses the isolation and culture of bovine stem cell-like cell lines.

Strelchenko, N. *et al.*, *Theriogenology 41*: 304 (1994) (Attachment 11) discloses the isolation and culture of bovine embryonic pluripotent cell lines.

Sukoyan, M.A. *et al.*, *Molec. Reprod. Develop. 33*: 418-431 (1992) (Attachment 12) discloses the isolation and culture of mink embryonic stem cell lines, and the production of chimeric mink.

Thompson, S. *et al.*, *Cell 56*: 313-321 (1989) (Attachment 13)

discloses the isolation and culture of mouse embryonic stem cell lines, and the production of chimeric mice.

Thompson, J.A. *et al.*, *Proc. Natl. Acad. Sci. USA 92*: 7844-7848 (1995) (Attachment 14) discloses the isolation and culture of a primate embryonic stem cell line.

Thompson, J.A., U.S. Patent No. 5,843,780, filed January 18, 1996, issued December 1, 1998 (Attachment 15) discloses the isolation and culture of a primate embryonic stem cell line.

Wakamatsu, Y. *et al.*, *Molec. Marine Biology Biotechnology 3*: 185-191 (1994) (Attachment 16) discloses the establishment of a pluripotent cell line derived from a fish.

In light of the above-cited literature, the Examiner has not provided objective evidence or sound technical reasons to doubt that, prior to the January 10, 1997 priority filing date of the present application, those of ordinary skill in the art could have cultured embryonic stem cells from multiple strains of mice and from non-mice species.

c. Only Routine Experimentation Would Have Been Required To Develop Serum-Free Cell Culture Media That Support the Expansion of Embryonic Stem Cells In Vitro

At pages 5-6 of the Office Action, the Examiner stated:

> The claims are also non-enabled as they broadly recite supplement ingredients to be used in the claimed culture methods and claimed products of manufacture, i.e., supplement ingredients which are capable of supporting growth of embryonic stem cells. While the specification

> disclose a particle [sic] medium formulation containing the supplement ingredients at specific concentrations, and provides evidence that such a medium formulation supports embryonic stem cell growth/differentiation, the specification does not disclose or provide evidence that the broadly claimed supplement ingredients at any concentration range support the growth/differentiation of embryonic stem cells. The specification fails to provide an enabling disclosure for how to make and use any and all combinations of media supplements, media formulations, and media useful for the claimed methods as the specification does not provide adequate guidance for the selection of appropriate serum-free medium supplements that would support the expansion/differentiation of embryonic stem cells as required of the claims. While the specification provides an explicit teaching regarding ingredients that could be used to make a medium appropriate to practice the claimed invention, the specification does not provide guidance as to which ingredients can be excluded from the formulation, and whether the concentrations of the remaining ingredients need optimization as a result of exclusion of particular ingredients.

Applicants respectfully disagree. One of ordinary skill in the art would not have practiced the claimed invention in a vacuum. Instead, the artisan would have had the benefit of Applicants' disclosure, in which Applicants have provided one of ordinary skill in the art with starting points, from which formula ingredients and concentrations may be optimized.

For example, Applicants have provided the ingredients and concentrations provided in Tables 1-3, at pages 27-30 of the present specification. Further, in the specification at page 22, lines 3-9, Applicants disclose:

> The concentration ranges within which ingredients are believed to support the growth of ES and other cells in culture are listed in Tables 1-3. These ingredients can be combined to form the cell culture medium supplement of the present invention. As will be readily apparent to one of ordinary skill in the art, the concentration of a given

> ingredient can be increased or decreased beyond the range disclosed and the effect of the increased or decreased concentration can be determined using only routine experimentation.

Serum-free medium development is an empirical art, and necessarily involves trial and error. However, simply because trial and error would have been required to have made a serum-free medium for use in the claimed methods, composition, and product of manufacture, that does *not* mean that the amount of trial and error required would have been *undue*. *See Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) ("Enablement is not precluded by the necessity for some experimentation such as routine screening.")

Appended hereto, collectively, as Attachment 17 are pages 80-81 of Freshney, R.I., "Culture of Animal Cells: A Manual of Basic Technique, Second Edition, Alan R. Liss, Inc., New York (1987) ("Freshney"). Freshney provides:

> As with medium and serum section, trial and error may be the only method to select the correct supplements. If a group of compounds is found to be effective in reducing serum supplementation, *the active constitutents [sic] may be identified by systematic omission of single components, and then their concentrations optimized.* [Ham, 1984].

Freshney at page 81, left column, first full paragraph (emphasis added) (bracketed citation to "Ham, 1984" in original).

Thus, Freshney supports Applicants' argument that only routine experimentation would have been required to practice the claimed methods. Freshney reflects that those of ordinary skill in the art of cell culture media development have long been accustomed to identifying active ingredients in new serum-free medium formulations by systematically omitting single components, and optimizing ingredient

concentrations. Those of ordinary skill in the art could have taken Applicants' disclosed medium formulation and developed a significant number of operative formulations containing fewer ingredients than are recited in Tables 1-3 of the present specification.

Those of ordinary skill in the art of cell culture media development have long been accustomed to identifying active ingredients in new serum-free medium formulations by systematically omitting single components, and optimizing ingredient concentrations. The art is replete with evidence that those of ordinary skill in the art could have taken Applicants' disclosed medium formulation and developed a significant number of operative formulations containing either fewer ingredients than are recited in the present specification.

For example, appended as Attachment 18 is copy of Jayme, D.W. and D.F. Gruber,[2] "Development of Serum-Free Media and Methods for Optimization of Nutrient Composition," in *Cell Biology: A Laboratory Handbook*, Celis, J.E., Ed., Vol. 1, Academic Press, San Diego, California (1994), pages 18-24 ("Jayme"). At page 21, first full paragraph, Jayme states, "[k]ey steps toward development of a serum-free formulation for the desired cell type include the following," and Jayme then provides a standard approach for identifying medium ingredients and concentrations.

Appended as Attachment 19 is a copy of Gruber, D.F. and D.W. Jayme,[3] "Cell and Tissue Culture Media: History and Terminology," in *Cell Biology: A Laboratory*

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[2] The authors are employees of Life Technologies, Inc., the assignee of the present application.

[3] The authors are employees of Life Technologies, Inc., the assignee of the present application.

Handbook, Celis, J.E., Ed., Vol. 1, Academic Press, San Diego, California (1994), pages 451-458 of the Appendices chapter ("Gruber"). Gruber teaches:

> The nutrient environment or "acceptable" cell growth is basically simple, yet specifically complex. The cellular milieu must address numerous physical and chemical factors, including temperature, osmotic pressure, hydrogen ion concentration (pH), and the presence of inorganic salts, essential (and nonessential) amino acids, vitamins, dissolved gasses, and other unidentified growth materials provided through supplementation(s). *Each of these interdependent requirements had to be assessed, independently and jointly, to ascertain optimal nutrient levels.*

Gruber at 453 (emphasis added).

Appended as Attachment 20 is Ham, R.G. and W.L. McKeehan, *Methods In Enzymology LVIII*: 44-93 (1979) ("Ham"). At pages 75-77, Ham describes a cell culture assay for use in determining which ingredients in a medium are required for cell growth. Ham reflects that the ability to systematically study individual ingredients was a skill possessed by those of ordinary skill in the art.

> *Systematic study* of the effects of individual variables requires specifically that all interactions be recognized and that all other significant variables be held constant *while the effects of each single variable or interacting group on cellular multiplication* are being studied.

Ham at 79, last full paragraph (emphases added). At page 92, Ham provides a generalized sequence of steps for minimizing serum requirements and for developing defined media.

In light of the above-cited literature, the Examiner has not provided objective evidence or sound technical reasons to doubt that those of ordinary skill in the art could have taken Applicants' disclosed medium formulation and developed a serum-

free medium containing fewer ingredients than are recited in Tables 1-3 of the present specification.

3. Summary

In summary, the reasons proffered by the Examiner fail to establish a *prima facie* case of non-enablement, because neither objective evidence nor sound scientific reasoning has been presented to show that the claimed invention is not enabled. In view of Applicants' application and knowledge possessed by one of ordinary skill in the art, the claimed invention is enabled. Given Applicants' disclosure, including the examples and figures in the present application, and the knowledge possessed by those of ordinary skill in the art when Applicants' application was filed, the claimed invention could have been practiced without undue experimentation.

Applicants respectfully request that this rejection be reconsidered and withdrawn.

C. With Respect to the Rejection of Claims 117-121, a Prima Facie Case of Non-Enablement Has Not Been Established

At page 7 of the Office Action, the Examiner stated:

> Claims 117-121, directed to differentiating the cells in serum-free culture are also not enabled as the specification clearly indicates, on page 44, that when the cells are plated on electrostatically charged plastic and allowed to attach, the embryoid bodies would not attach without the addition of 1% FBS to supply undefined attachment factors. Once attached, the differentiated cells that grew out of the embryoid bodies were quite different than those seen in FBS-supplemented medium. While the specification indicates that cells can be induced to

> differentiate to at least cardiac cells, it is apparent from the specification that serum is required to effect differentiation. Thus, in view of the teachings in the specification, and the absence of guidance as to serum-free ingredients which can be used to replace the 1% FBS, one of skill in the art would not have had a high expectation of successfully differentiating embryonic stem cells under serum-free conditions without undue experimentation.

Applicants respectfully traverse this rejection. A *prima facie* case of non-enablement has not been established.

Claims 117 and 118 are directed to a method of causing embryonic stem cells to differentiate into a particular type of cell in serum-free culture. Claim 119 depends from claim 117 or 118, and recites that the method further comprises seeding embryonic stem cells upon a layer of feeder cells. Claim 120 depends from claim 117 or 118, and recites that one or more growth factors that prevents differentiation of embryonic stem cells is added to the culture medium. Claim 121 depends from claim 117 or 118, and recites that one or more growth factors that facilitates differentiation of embryonic stem cells is added to the culture medium.

No evidence or sound technical reasons have been provided as to why, after reading the present specification, one of ordinary skill in the art would not have been able to practice the methods of claims 117-121 without undue experimentation. The Examiner's statement that "when the cells are plated on electrostatically charge plastic and allowed to attach, the embryoid bodies would not attach without the addition of 1% FBS to supply undefined attachment factors" does not create a *prima facie* case of non-enablement.

In the example at page 44 of the present specification, 1% serum was used to provide attachment factors. At page 44 of the specification, lines 13-15, Applicants

explained that "[i]t is expected that purified attachment factors can be substituted for the one percent serum that was used to supply such factors." The Examiner has failed to provide any evidence or technical reasons why it would have required undue experimentation to facilitate attachment using purified attachment factors.

The Examiner's statement that "[o]nce attached, the differentiated cells that grew out of the embryoid bodies were quite different than those seen in FBS-supplemented medium" does not support a *prima facie* case of non-enablement. It is irrelevant whether differentiated cells that emerge from embryoid bodies attached in serum-containing medium might be different from cells that emerge from embryoid bodies attached in serum-free medium. What is relevant is whether any evidence or reasons have been given to doubt that the methods of claims 117-121 are not enabled.

The reasons proffered by the Examiner fail to establish a *prima facie* case of non-enablement, because neither objective evidence nor sound scientific reasoning has been presented to show that the claimed invention is not enabled. In view of Applicants' application and knowledge possessed by one of ordinary skill in the art, the claimed invention is enabled. Given Applicants' disclosure, including the examples and figures in the present application, and the knowledge possessed by those of ordinary skill in the art when Applicants' application was filed, the claimed invention could have been practiced without undue experimentation.

Applicants respectfully request that this rejection be reconsidered and withdrawn.

VII. The Rejection Of Claims 91-104 and 108-126, Under 35 U.S.C. § 112, Second Paragraph, Must Be Withdrawn

A. The Rejection of Claims 91, 98-103, 122 and 123 Must Be Withdrawn

At page 7 of the Office Action, that Examiner rejected claims 91, 98-103,122 and 123 as allegedly indefinite for reciting the phrase "is capable of." Applicants respectfully traverse this rejection.

Claims 91, 98-103,122 and 123 have been amended to recite "supporting." As a result of this amendment, it is believed that the ground for this objection is moot.

Applicants respectfully request that this rejection be reconsidered and withdrawn.

B. The Rejection of Claims 108, 109 and 111 Must Be Withdrawn

At pp. 7-8 of the Office Action, the Examiner rejected claims 108,109 and 111 as allegedly indefinite for reciting the phrase "controlling or preventing." Applicants respectfully traverse this rejection.

According to the Examiner, it is allegedly unclear whether Applicants are using the terms "controlling" and "preventing" interchangeably, or if there are distinct culture conditions that allow for the control of differentiation, compared to culture conditions that prevent differentiation. The terms "controlling" and "preventing" are not synonymous or interchangeable. "Controlling" differentiation can relate to the *rate* at which an embryonic stem cell is allowed to differentiate. In contrast, "preventing" differentiation relates to preventing embryonic stem cells differentiating at all.

Applicants respectfully request that this rejection be reconsidered and withdrawn.

C. The Rejection of Claims 117 and 118 Must Be Withdrawn

At page 8 of the Office Action, the Examiner rejected claims 117 and 118 as allegedly indefinite because they recite the phrase "or changing culture conditions." According to the Examiner, it is allegedly unclear how culture conditions should be changed such that differentiation is induced. Applicants respectfully traverse this rejection.

Those of ordinary skill in the art were familiar with a number of approaches in which culture conditions could be changed. Such approaches include, but are not limited to, adding ingredients to the cell culture medium, removing ingredients from the cell culture medium, changing atmospheric conditions in the cell culture system, providing the cells with fresh medium, and providing the cells with conditioned medium.

Applicants respectfully request that this rejection be reconsidered and withdrawn.

D. The Rejection of Claim 124 Must Be Withdrawn

At page 8 the Office Action, the Examiner rejected claim 124 as allegedly indefinite for reciting the phrase "recombinant protein embryonic stem cells." Applicants respectfully traverse this rejection.

Claim 124 has been amended to recite "recombinant protein in embryonic stem cells." As a result of this amendment, it is believed that the ground for this objection is moot.

Applicants respectfully request that the rejection of claim 124 be reconsidered and withdrawn.

VIII. The Rejections Under 35 U.S.C. § 102 Must Be Withdrawn

A. The Rejections of Claim 101 Must Be Withdrawn

At page 8 of the Office Action, the Examiner rejected claim 101, under 35 U.S.C. § 102(b), as allegedly anticipated by the Sigma Chemical Company catalog (1994) ("Sigma"). Applicants respectfully traverse this rejection. As amended, claim 101 recites a product of manufacture with a third container means that contains embryonic stem cells. Sigma fails to teach the product of manufacture of claim 101, because Sigma fails to teach embryonic stem cells. Applicants respectfully request that this rejection be reconsidered and withdrawn.

At page 9 of the Office Action, the Examiner rejected claim 101, under 35 U.S.C. § 102(b), as allegedly anticipated by the GibcoBRL Life Technologies Catalogue (1993-1994) ("GiboBRL"). Applicants respectfully traverse this rejection. GibcoBRL fails to teach the product of manufacture of claim 101, because GibcoBRL fails to teach embryonic stem cells. Applicants respectfully request that this rejection be reconsidered and withdrawn.

B. The Rejections of Claims 102 and 103 Must Be Withdrawn

At page 9 of the Office Action, the Examiner rejected claims 102 and 103, under 35 U.S.C. § 102(b), as allegedly anticipated by Ponting, U.S. Patent No. 5,405,772 ("Ponting"). Applicants respectfully traverse this rejection. As amended, claims 102 and 103 recite a product of manufacture with a second container means that contains embryonic stem cells. Ponting fails to anticipate claims 102 and 103, because Ponting fails to teach embryonic stem cells. Applicants respectfully request that this rejection be reconsidered and withdrawn.

At page 9 of the Office Action, the Examiner rejected claim 102 and 103 as allegedly anticipated by Cleveland, U.S. Patent No. 4,767,704 ("Cleveland"). Applicants respectfully traverse this rejection. Cleveland fails to anticipate claims 102 and 103, because Cleveland fails to teach embryonic stem cells. Applicants respectfully request that this rejection be reconsidered and withdrawn.

C. The Rejection of Claims 101-103 Must Be Withdrawn

At page 10 of the Office Action, the Examiner rejected claim 101-103, under 35 U.S.C. § 102(b), as allegedly anticipated by Ramos, WO 92/05246 ("Ramos"). Applicants respectfully traverse this rejection. As amended, claims 101-103 recite a product of manufacture with a container means that contains embryonic stem cells. Ramos fails to teach the product of manufacture of claims 101-103, because Ramos fails to teach embryonic stem cells. Applicants respectfully request that this rejection be reconsidered and withdrawn.

D. The Rejections of Claim 104 Must Be Withdrawn

At page 10 of the Office Action, the Examiner rejected claim 104, under 35 U.S.C. § 102(b), as allegedly anticipated by Sigma. Applicants respectfully traverse this rejection. Claim 104 depends multiply from claims 101-103. As amended, claims 101-103 recite a product of manufacture that contains embryonic stem cells. Sigma fails to teach the product of manufacture of claim 104, because Sigma fails to teach embryonic stem cells. Applicants respectfully request that this rejection be reconsidered and withdrawn.

At page 10 of the Office Action, the Examiner rejected claim 104 as allegedly anticipated by GibcoBRL. Applicants respectfully traverse this rejection. GibcoBRL fails to teach the product of manufacture of claim 104, because GibcoBRL fails to teach embryonic stem cells.

Applicants respectfully request that this rejection be reconsidered and withdrawn.

IX. The Rejections Under 35 U.S.C. § 103 Must Be Withdrawn

At page 12 of the Office Action, the Examiner rejected claims 101-104, under 35 U.S.C. § 103, as allegedly obvious over

(1) Cleveland or Ponting or Ramos, each taken with

(2) Maurer, H.R., "Towards Chemically-defined, Serum-free Media for Mammalian Cell Culture," Chapter 2 in: *Animal cell culture: a practical approach*, Freshney, R.I., Ed., IRL Press, pub., pp. 13-31 (1986) ("Maurer"), and either

(3) Sigma, or

(4) GibcoBRL.

Applicants respectfully traverse these rejections. A *prima facie* case of obviousness has not been established.

As amended, claims 101-103 recite a product of manufacture with a container means that contains embryonic stem cells. Claim 104 depends multiply from claims 101-103, and recites that the product of manufacture is in a frozen state.

None of the art cited by the Examiner relates to embryonic stem cells. Cleveland, Ponting, Ramos, Maurer, Sigma, and GibcoBRL are each silent about embryonic stem cells. Thus, none in any of various combinations of art proposed by the Examiner would have suggested the product of manufacture of any of claims 101-104.

Applicants respectfully request that this rejection be reconsidered and withdrawn

Conclusion

All of the stated grounds of objection and rejection have been properly traversed. Applicants therefore respectfully request that the Examiner reconsider and withdraw all of the outstanding objections and rejections.

Applicant believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will

expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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